PHARMA-ZENTRALE GMBH

ANALYSENZERTIFIKAT

Produkt: ***Escherichia coli*** **Stamm NISSLE 1917 (DSM 6601) Suspension**
Ch.-B.: **240060**

Prüfparameter	Anforderung	Prüfergebnis
Gleichförmigkeit der Masse	Prüfung muss den Vorgaben der Spezifikation entsprechen	entspricht der Anforderung Mittelwert = 15,16 g Spanne: 14.70 bis 15,74 g
Gleichförmigkeit des Gehaltes	Prüfung muss den Vorgaben der Spezifikation entsprechen	entspricht der Anforderung Mittelwert = 1,001 EE Spanne: 0,981 EE bis 1,009 EE
Resuspendierbarkeit	Prüfung muss den Vorgaben der Spezifikation entsprechen	homogene Suspension; kein Sediment sichtbar
Dichte	1,00 ± 0,05 g/ml	1,002 g/ml
pH-Wert	7,0 ± 1,0	6,78
Leitfähigkeit	7 – 11 mS/cm	8,80 mS/cm
Prüfung auf Identität *E. coli* Stamm NISSLE 1917 (DSM 6601)	Biotest ID-GNI-System Code: 2560654	Code: 2560654
Mikrobiologische Reinheit	*Staph. aureus* n.n./1ml *Ps. aeruginosa* n.n./1ml Salmonellen n.n./10ml aerobe Bakterien n.n./0,1ml Pilze n.n./0,1ml anaerobe Bakterien n.n./0,1ml	nicht nachweisbar nicht nachweisbar nicht nachweisbar nicht nachweisbar nicht nachweisbar nicht nachweisbar
Hämolyse	keine Hämolyse nachweisbar	Hämolyse nicht nachweisbar
Keimzahl	$10^8 – 10^9$ lebensfähige Bakterien (KBE) *E. coli* Stamm NISSLE 1917 (DSM 6601) / ml Suspension	$1,1 \times 10^9$ KBE/ml

n.n. = nicht nachweisbar EE = Extinktionseinheiten

Prüfdatum: Januar 2003
Freigabedatum: 20.01.03

Herdecke, den 20.01.2003

Dr. Holzkamp

Datei: M:\QK\Firmenbezogene_Dokumente\PharmaZentrale\EcoliSuspension\240060.doc

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Mutaflor® Suspension

ARDEYPHARM

1. Bezeichnung des Arzneimittels

MUTAFLOR® Suspension

Wirkstoff: E. coli Stamm Nissle 1917

2. Verschreibungsstatus / Apothekenpflicht

Apothekenpflichtig

3. Zusammensetzung des Arzneimittels

3.1 Stoff- oder Indikationsgruppe

Darmtherapeutikum

3.2 Arzneilich wirksame Bestandteile nach Art und Menge

1 ml enthält:
Biomasse mit 10^8 lebensfähigen Bakterien E. coli Stamm Nissle 1917

3.3 Weitere Bestandteile nach der Art

Gereinigtes Wasser, Natriumchlorid, Kaliumchlorid, Magnesiumsulfat, Calciumchlorid, Magnesiumchlorid

4. Anwendungsgebiete

Störungen der Dickdarmflora und deren Folgezustände, Diarrhoe, Colitis, Obstipation, Meteorismus, Ekzeme, Allergien. Neuaufbau der Darmflora nach Schädigung durch Antibiotika, Sulfonamide und Bestrahlungen. Harnwegsinfektionen. Aktivierung körpereigener Abwehrkräfte.

5. Gegenanzeigen

Keine bekannt.

6. Nebenwirkungen

Es sind keine Nebenwirkungen bekannt.

7. Wechselwirkungen mit anderen Mitteln

Antibiotika und Sulfonamide können die Wirksamkeit von MUTAFLOR Suspension einschränken.

8. Warnhinweise

Keine

9. Wichtigste Inkompatibilitäten

Keine bekannt.

10. Dosierung mit Einzel- und Tagesgaben

Säuglinge: 1 ml täglich einnehmen.

Kinder: 1–3 ml täglich einnehmen.

Erwachsene: 1–5 ml täglich einnehmen.

11. Art und Dauer der Anwendung

Das Einzelbehältnis wird vom Block abgerissen. Vor Anwendung kräftig schütteln und den Verschluß durch Drehen entfernen. Die Suspension kann direkt aus dem Behältnis in den Mund geträufelt werden, bei Säuglingen vor dem Trinken, bei Kindern und Erwachsenen nach einer Mahlzeit.

Die Dauer der Anwendung richtet sich nach dem Verlauf der Erkrankung. Für die wirkungsvolle Besserung der Symptome chronischer Erkrankungen ist eine Anwendung von mindestens 6 Wochen zu empfehlen. Bei schon jahrelang bestehenden Erkrankungen ist eine Anwendungsdauer von mehreren Monaten angezeigt.

12. Notfallmaßnahmen, Symptome und Gegenmittel

Unverträglichkeiten sind bislang nicht bekannt geworden.

Bei auftretenden Überempfindlichkeitsreaktionen ist das Präparat abzusetzen.

13. Pharmakologische und toxikologische Eigenschaften und Angaben über Pharmakokinetik und Bioverfügbarkeit, soweit diese Angaben für die therapeutische Verwendung erforderlich sind

13.1 Pharmakologische Eigenschaften

MUTAFLOR Suspension enthält einen definierten, nicht-pathogenen Stamm von Escherichia coli (Stamm Nissle 1917) in lebensfähiger Form. Die Wirkungen von MUTAFLOR wurden in in-vitro- und in-vivo-Experimenten sowie in klinischen Studien nachgewiesen. Dabei wurden folgende Eigenschaften und Wirkprinzipien ermittelt:

- Der Stamm bildet antimikrobielle Substanzen, auf denen der Antagonismus gegen pathogene Keime beruht.
- Der Stamm produziert kurzkettige Fettsäuren, die für den Energiehaushalt der Kolonmukosa von Wichtigkeit sind, die Kolonmotilität und -durchblutung anregen und die Natrium- und Chloridabsorption fördern.
- Der MUTAFLOR-Stamm ist in der Lage, verschiedene Kohlenhydrate, Zuckeralkohole und andere Substrate unter Sauerstoffverbrauch abzubauen. Dadurch wird ein anaerobes Milieu im Kolon erzeugt.
- Mit Hilfe spezieller Haftorganellen (normale Typ-I-Fimbrien) kann sich der Stamm an der Darmwand anheften. Der Stamm ist gut beweglich, was einen Vorteil für die Besiedlung des Dickdarms darstellt.
- Durch MUTAFLOR wird sowohl das spezifische als auch das unspezifische Immunsystem stimuliert.

Spezifisches Immunsystem:

Neugeborene zeigen nach Kolonisierung mit dem E. coli Stamm Nissle 1917 eine signifikante Erhöhung der IgA- und IgM-Spiegel in Stuhlfiltraten und Blutserum. Aus Einzeluntersuchungen ergaben sich Hinweise für eine Erhöhung von IgA im Speichel.

Unspezifisches Immunsystem:

In-vitro-Versuche ergaben eine signifikante Steigerung der sekretorischen Leistungen von Mausmakrophagen. Die Produktion von Interleukin 6 (= Interferon b_2) und Sauerstoffradikalen wurde signifikant erhöht. Zusätzlich wurde eine erhöhte Produktion von Tumornekrosefaktor (TNF) nach Induktion der Makrophagen durch E. coli Stamm Nissle 1917 nachgewiesen.

13.2 Toxikologische Eigenschaften

Der Keim wurde in einer Reihe von mikrobiologischen, molekularbiologischen, serologischen und biochemischen Experimenten auf toxische oder pathogene Eigenschaften geprüft. Er hat keine toxischen oder pathogenen Eigenschaften, Er bildet keine Enterotoxine, ist nicht enteroinvasiv, zeigt keine pathogenen Adhäsionsmarkmale, bildet keine Hämolysin, ist nicht serumresistent, zeigt keine uropathogenen Eigenschaften und ist empfindlich gegenüber gängigen Antibiotika.

14. Sonstige Hinweise

Bei schweren Diarrhöen, besonders im Säuglingsalter, besteht die Gefahr einer Exsikkose. Deshalb sollte auch bei der MUTAFLOR-Therapie eine genügende Rehydrierung erfolgen.

Für Diabetiker: Es ist keine Berechnung von BE erforderlich.

15. Dauer der Haltbarkeit

Verfalldatum!
MUTAFLOR enthält lebensfähige, physiologische E.coli Bakterien, die nur eine begrenzte Zeit haltbar sind.

Nach Ablauf des Verfalldatums soll MUTAFLOR Suspension nicht mehr angewendet werden.

16. Besondere Lager- und Aufbewahrungshinweise

Nicht über 8°C lagern!

17. Darreichungsformen und Packungsgrößen

Originalpackung mit 5 x 1 ml (N1)
Originalpackung mit 25 x 1 ml (N2)
Originalpackung mit 5 x 5 ml (N1)
Originalpackung mit 25 x 5 ml (N2)

18. Stand der Information

November 1997

19. Name oder Firma und Anschrift des pharmazeutischen Unternehmers

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German Patent and Trade Mark Office

German Patent and Trade Mark Office - 80297 Munich

Munich, January 30th, 2004
Phone: (089) 2195 - 4770
Ref.-No.: 197 51 907.5 - 41
Applicant's No.: 4083865
Pharma-Zentrale GmbH

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Your Ref.: Pt. 78/97 Dr.Si/ge

Harmsen & Utescher
Received February 9th, 2004

Issue resolution

On the application 197 51 907.5 - 41 of Mr, Mrs, Company

Pharma-Zentrale GmbH, 58313 Herdecke, Germany;

a patent, valid as of November 23rd, 1997,

named

Use of Escherichia coli strain DSM 6601 for the treatment of diarrhea in veterinary medicine

with the documentation according to the enclosed photocopy of form P2480, which is a part of this resolution,

is issued.

The patent is given the number 19751907.

We point out the statement of rights of appeal which is printed overleaf.

Examining board for class A61K

Thielemann
(signed)

Authorized by the Hamm Appellate Court to certify the correctness and completeness of translations from the German to the English and from the English to the German language.

Declaration of receipt

Print documents for the patent specification

	10. 1.	10. 2.	10. 3.	10. 4.	
	Valid page / column	Reception date (or disclosure specification)	Changes as per	Editing has been done to page / column	
Description with denomination	p. 1 – 10	Nov. 22nd, 1997	O		
			O		
			O		
			O		
	Valid number	Reception date (or disclosure specification)	Changes as per	Editing has been done to number	
Patent claims (with one claim: enter "1")	1	Dec. 05th, 2003	O		
			O		
			O		
			O		
	Valid figure number	Reception date (or disclosure specification)	Changes as per	Editing has been done to figure number	Clues to the kind of editing
Figures (with one figure: enter "1")			O		
			O		
			O		

1) Notification / Annex to Notification as of _____
2) Petition as of _____
3) Agreements of the hearing dated _____
4) _____
5) _____ have been considered.

10. 5. A summary, comprising a figure if applicable, must be printed in case a **disclosure specification is not being published** or if the **disclosure specification has been published without a summary**.

Examining board for class A61K

(signed as of Dec. 19th, 2003)

_____
Signature of Inspector, Date

Ref.-No.: P 197 51 907.5 - 41

P 2480
9.96

This is to certify the correctness and completeness of the foregoing translation from the German language.

Sprockhövel — Place
9/3/2004 — Date

Signature

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Use of Escherichia coli strain DSM 6601 for the treatment of diarrhea in veterinary medicine

The invention relates to the use of Escherichia coli strain DSM 6601 for the prevention and treatment of diarrhea caused in animals by microbes.

Diarrhea in humans or animals is understood as frequent discharge of fluid feces for various causes, such as an allergic reaction to certain foods, but mostly as a reaction to microorganisms, especially bacteria, fungi or viruses or the toxins thereof. Because of great water and salt loss, prolonged diarrhea may lead to serious complications and even death. Severe diarrhea therefore always requires treatment, especially in the case of young or already weakened humans or animals.

In a large proportion of cases, diarrhea is caused by bacteria that do not belong to the normal microorganisms of the microflora of the affected body. Certain pathogenic strains of Escherichia coli, Salmonella and Shigella in particular play a major role in this regard. Diarrhea can be caused not only by bacteria, however, but also by infections with viruses, especially coronaviruses and rotaviruses, or by fungi, which in most cases are members of the Eumycota. Whereas diarrhea caused by bacteria can usually be combated quite effectively now by administration of sorbents or nonsystemic sulfonamides or antibiotics, pharmaceuticals which can be used successfully in viral infections have not yet been available, and hardly any pharmaceuticals are effective against colonization of the gastrointestinal tract with fungi.

Fungi are defined as a polyphylogenetic group of chlorophyll-free, heterotrophic and eukaryotic organisms, which can be single-celled or multiple-celled and whose reproduction proceeds with mitosis and meiosis, as for all eukaryotes, and by the formation of sexual or asexual spores or even by budding. The single-celled fungi that propagate only by budding are frequently grouped together as yeasts, even though the yeasts represent a conglomerate of different classes. Almost all Eumycota, or in other words the true fungi, pass through two or more morphologically distinguishable stages during their individual development, namely as teleomorphs, in which the spores are formed after meiosis, and one or more stages as anamorphs, in which spore formation is not associated with meiosis. Fungi whose teleomorphs are unknown or which have the lost the ability to form such are grouped together as Fungi imperfecti. Exact botanical classification is still unresolved for many of the fungi.

Because of their lack of chlorophyll, all fungi obtain their nutrition heterotrophically by degradation of organic substances; they are therefore saprophytes. In medicine a distinction is made between the opportunists, or in other words saprophytes which can become pathogenic rarely and only under strictly defined conditions. They include, for example, Candida and Aspergillus. Pathogenic saprophytes live a normal saprophytic existence outside the body, but they must be regarded as pathogenic for humans or animals under all circumstances if an infection has developed. The fungi also include obligate parasites, which cannot vegetate outside the host organism and which are found only in humans or animals; they include most dermatophytes.

One substantial difference between bacteria and fungi is based on the fact that fungi, in contrast to bacteria, are eukaryotes, meaning they possess a nucleus and membrane and, again in contrast to bacteria, they also possess mitochondria; another on the fact that the cell wall consists of chitin and/or cellulose, whereas the cell wall of the bacteria is formed from mucopeptides. This also explains why most antibiotics which are effective for bacteria fail for fungi, since in many known antibiotics the bacterial wall or the mitochondria are the main points of attack of these agents.

Some antibiotics are also known which may also be used for systemic and not merely topical treatment of fungal infections and which are characterized by a polyene structure, such as amphotericin B, griseofulvin, natamycin and nystatin. Synthetic systemic antimycotics are flucytosin and a series of azole derivatives such as ketoconazole and miconazole or fluconazole and itraconazole.

Whereas the exact mechanism of action of the polyene antibiotics is not yet known in all details, the synthetic azole derivatives act on the ergosterol synthesis in the cell membrane and thus influence the permeability of the cell wall.

A disadvantage of all antimycotics known heretofore that may also be used systemically, is that they usually act not as fungicides but merely as fungistats, thus necessitating a relatively long treatment period. Furthermore, cross resistances develop, both to the polyene antibiotics and to the azole compounds. In addition, finally, the price of these products is relatively high, thus hindering broad application in veterinary medicine.

Therefore, a further need exists for veterinary pharmaceuticals which are capable of effectively combating diarrhea caused in animals substantially by pathogenic fungi or even by the co-involvement thereof.

Completely surprisingly, it has now been discovered that such diarrhea can be effectively combated by using Escherichia coli strain DSM 6601, even when otherwise conventional medication with compounds having antifungal activity has failed.

Escherichia coli, abbreviated hereinafter as E. coli, exists in numerous varieties, which differ as regards capsule antigens, surface antigens and flagella antigens and can therefore be subdivided into numerous serological types. Classification by serotypes, however, does not provide any indication of the different virulence of the pathogens. Representatives of one and the same serotype can have different pathogenic potential both in the human and in the animal body, ranging in the extreme case from avirulent to highly pathogenic. It is known, however, that E. coli strain DSM 6601 is rated as nonpathogenic to humans or animals. As an example, this strain is used in human medicine as a substitution preparation in infectious intestinal diseases due to Salmonella or Shigella, both in acute and chronic cases. This E. coli strain is also used successfully in substitution therapy for other disorders of the intestinal flora, for example after antibiotic treatment or irradiation. It has not been unequivocally clarified whether what actually happens is that this special E. coli strain merely displaces the pathogenic bacterial strains, including the corresponding variants of E. coli or Proteus, thus causing a reduction in the toxins, or whether the metabolic products of E. coli strain DSM 6601 have a therapeutic effect in their own right.

Starting from these known explanations of the mechanism of action of living E. coli microorganisms, however, it could in no way be anticipated that treatment with these living cultures would have surprisingly extensive efficacy toward infection of the intestinal tract of animals with fungi, and in this context especially yeasts. Certainly apathogenic E. coli strains were occasionally used in veterinary medicine in the fifties for diseases of cattle or pigs, sometimes also involving diarrhea, but the objective was therapy for nutritional disorders in piglets (Fischer, W., Experiences of a practicing veterinarian in the treatment of sick piglets from 1945 to 1950; Dissertation, Munich University 1950), or the treatment of Semper's disease in cattle, which according to the results of this publication was obviously based on deficient nutrition due to the geology of the region (Häfele W., "Semper's disease", a nutritional and developmental disorder of cattle in the Upper Black Forest in the vicinity of St. Blasien; Dissertation, Vet. Med. Animal Clinic, Munich University 1952). These early attempts to use certain strains of E. coli experimentally for special diseases of pigs or cattle were entirely isolated, and not once did they provide the motivation to undertake further experiments of this type in diarrhea caused by bacteria. All the more surprising was the discovery that E. coli strain DSM 6601 exhibits a surprising effect even in intestinal diseases caused exclusively or substantially by fungi, since fungal infections are particularly difficult to combat, especially when the mucous membranes of the intestine are affected, since the preparations used for bacterial infections have practically no effect.

Heretofore surprisingly little has been known about the normal intestinal microflora in various animal species, but infestation of the gastrointestinal tract with fungi and especially with yeasts must always be regarded as a pathological process.

The surprisingly rapid efficacy of the treatment with E. coli strain DSM 6601 suggests that the effect of this treatment does not depend or does not depend only on substitution of the fungal flora by a healthy bacterial microflora, but instead that the strain contributes largely to an increase in the body's endogenous defense mechanisms, presumably because the metabolic products of this strain have a considerable immuno-stimulating effect.

The invention will be explained in more detail hereinafter by means of an example:

Example:

In a dairy farm in Saxony, which possessed on average a stock of 1480 milk cows, 600 heifers and young cows as well as 185 calves, the calf stock was characterized until early January 1997 by very good rearing results and low morbidity and mortality. Manifest gastric and intestinal diseases with diarrhea were extremely infrequent. Losses of calves were always below the 3% limit relative to the number of live-born calves.

By late January 1997 a sudden increase in the incidence of diarrhea among the stock, with clinically pronounced symptoms of gastroenteritis, was observed. The disease began among the calves aged from seven to eleven days, was of various duration and was characterized by a morbidity of more than 90% and a mortality of more than 10%. The course of this disease was marked by the following symptoms: initially greenish-yellowish diarrhea with the consistency of thick paste, after which, in the further course of the disease, the feces became slimy, increasingly thin and mostly watery. The body temperature readings were slightly elevated at the beginning of the disease (39.7 to 39.9 °C), but then dropped rapidly in the further course and reached only lower limits, some in the range of 37.8 to 37.5 °C. The diseased calves exhibited pronounced aversion to suckling and increasing weakness, ultimately just lying down in one place in the further course of the disease. Seriously sick animals had to be forcibly fed.

After a period of three to nine days of illness, the losses became increasingly worse. The autopsies performed consistently revealed the most prominent findings, namely fluid, flocculent to slightly bloody intestinal contents and advanced, ulcerative inflammation of the omasum and abomasum. In the month of April 1997, the incidence of the disease reached values of almost 100% and the mortality rose to more than 15%.

The microbiological studies of the gastric and intestinal contents and of the internal organs were initially without specific results until February 1997, when yeasts of the genus Candida (C. glabrata and C. albicans) were isolated from the gastric and intestinal contents of two dead calves. Intestinally pathogenic E. coli were not found. These yeasts were detected in all further autopsies of dead calves and in the studies of fecal specimens of calves and their mothers. Further specific studies of fecal and colostric specimens of the cows and heifers yielded identical results. Supplementary studies for viruses, namely coronaviruses and rotaviruses, as well as for cryptosporidae revealed coronavirus particles only in two cases and rotaviruses in one case among dead calves.

The origin of the yeasts remained inexplicable for a prolonged period of time, until March 1997, when in a study of brewery residues used as fodder it was possible to detect yeasts (with Candida glabrata dominating) in a concentration of 3.6×10^7 CFU per g of fodder.

The therapeutic measures immediately performed extensively, such as dietary drinks, antiphlogistics, antidiarrhoic substances, electrolytes and cardiovascular agents led only to unsatisfactory results. When sulfonamides and antibiotics were used, the animals died six to eight hours after application. Once these therapies had proved unsuccessful, therapeutics containing humic acids as the active agents were used. Even these actions did not achieve any perceptible improvements of the course of the disease.

Of the 236 calves born alive in two months from 24 April to 23 June 1997, all animals became sick and 41 died in this period, corresponding to a proportion of 17.4%. Of those, 29 animals, corresponding to 70.7% of the losses, died from diarrhea; eight animals, corresponding to 19.5% of the losses, died from diarrhea with concomitant bronchopneumonia; and four animals, corresponding to 9.8% of the losses, died from other causes.

In the period from 25 June 1997 to 04 September 1997, a suspension of living E. coli strain DSM 6601 was then administered to 300 newborn calves in an amount of 15.0 ml per calf per day – corresponding to ... CFU/ml. This dose was independent of the weight and age of the animal and was administered orally, specifically for a duration of 10 to 13 days after birth. In the period from 24 June to 08 July 1997, 61 calves were treated in this way. Two exhibited diarrhea, corresponding to a morbidity of 3.3%. None of the animals died of this disease.

In the period from 09 July to 23 July 1997, 49 calves were treated correspondingly. None of these suffered from gastroenteritis with diarrhea. The mortality due to this disease was therefore 0%.

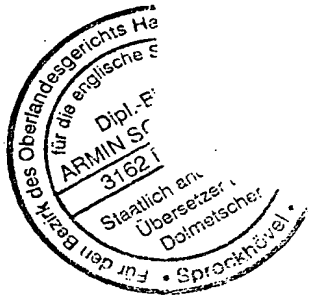
In the period from 23 July to 06 August 1997, 64 calves were treated. Two exhibited diarrhea, corresponding to a morbidity of 3.1%. None of the animals died of this disease, and so for this period the mortality was 0%.

After all existing therapeutic options had been exhausted, the occurrence of diarrhea due to gastroenteritis in suckling calves was prevented almost completely by the preventive and therapeutic application of a suspension of E. coli strain DSM 6601.

It was possible to considerably reduce the use of other pharmaceuticals and dietetics, and so the costs expended for these substances were lowered by 70%.

Claims

1. The use of Escherichia coli strain DSM 6601 for producing pharmaceuticals for the prevention and treatment of diarrhea caused in the gastrointestinal tract of animals by microbes, with involvement of pathogenic fungi.



This is to certify the correctness and completeness of the foregoing translation from the German language.

Sprockhövel — Place

8/3/2004 — Date

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